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Platinum Complexes of Diaminocarboxylates and Their Ethyl Ester Derivatives: Antitumor Activity and Interaction with Deoxyribonucleic Acid

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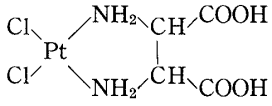
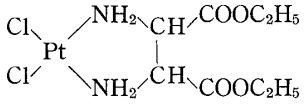
The platinum complexes of diaminocarboxylates (charged complexes) and their ethyl ester derivatives (non-charged complexes) were prepared and examined for antitumor activity. The non-charged complexes are antitumor-active, while the charged complexes are inactive. The interaction of deoxyribonucleic acid (DNA) with the charged and non-charged complexes was examined by means of ultraviolet (UV) difference spectroscopy. Since the UV difference spectral patterns obtained in this work were almost the same, the binding mode of the charged complexes to DNA seems to be the same as that of the non-charged complexes. However, the presence of negative charge lowers the reactivity of the platinum complexes with DNA. The antitumor-inactivity of the charged complexes may be due to a combination of their reduced ability to permeate into cells and the lower reactivity with DNA.

Keywords——platinum complex; antitumor activity; structure-activity relationship; interaction of metal complex with DNA; diaminocarboxylate metal complexes

In connection with the structure-activity relationships of platinum complexes, only neutral species have been reported to possess antitumor activity.[2,3] The miscellaneous charged complexes tested so far have all had no antitumor activity. However, these complexes may not have satisfied other necessary conditions for the appearance of antitumor activity. For example, K[Pt(glycine)Cl_2] has a *cis*-leaving group of intermediate leaving ability, but the donor atoms on the non-leaving ligand are oxygen and nitrogen.[4,5]



Pt(dasa)Cl_2



Pt(dasa-ester)Cl_2

Chart 1

In this work, the platinum complexes of diaminocarboxylates (*meso*-1,2-diaminosuccinic acid, dasa; L-1,2-diaminopropionic acid, dapa; and L-1,3-diaminobutyric acid, daba) and their ethyl ester derivatives (diethyl 1,2-diaminosuccinate, dasa-ester; ethyl 1,2-diaminopropionate, dapa-ester; and ethyl 1,3-diaminobutylate, daba-ester) were prepared. The necessary conditions for the appearance of antitumor activity are satisfied in these platinum complexes except for the problem of charge. Generally, deoxyribonucleic acid (DNA) is believed to be the primary binding site of the antitumor-active platinum complexes. If platinum complexes

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2) M.J. Cleare, *Coord. Chem. Rev.*, **12**, 349 (1974).
3) M.J. Cleare and J.D. Hoeschele, *Platinum Metal Rev.*, **17**, 2 (1973).
4) M.J. Cleare, *Bioinorg. Chem.*, **2**, 187 (1973).
5) H.K.V. Leh and W. Wolf, *J. Pharm. Sci.*, **65**, 315 (1976).

are transported into cells by passive diffusion, neutral species should penetrate the cell membrance more easily than charged species, and this may be the reason for the inactivity of certain charged complexes. We therefore used UV difference spectroscopy to examine the interaction of these platinum complexes with DNA.

Experimental

Preparation of the Platinum Complexes——Equimolar amounts of dasa (10 mmol) and K_2PtCl_4 (10 mmol) were mixed in 0.2 M HCl solution (500 ml) and boiled until the dasa was dissolved. The solution was evaporated to dryness, and the residual solid was recrystallized from 0.1 M HCl solution. Yellow crystals of Pt(dasa)Cl_2 were obtained.

Equimolar amounts of dapa·HCl (10 mmol) and K_2PtCl_4 (10 mmol) were dissolved in 100 ml of water and then the solution was allowed to stand for 24 hr at room temperature. Granular dark red crystals and fine yellow crystals that precipitated out were separated by decantation with stirring. The solution containing the fine yellow crystals was filtered and the needles were collected on a sintered-glass filter. Recrystallization from 0.1 M HCl solution gave yellow needles of Pt(dapa)Cl_2.

Equimolar amounts of daba·HCl (10 mmol) and K_2PtCl_4 (10 mmol) were dissolved in 100 ml of water and then the solution was adjusted to pH 2.0—3.0. The resulting solution was warmed on a water bath for several hours. After standing at room temperature for a day, the pale yellow crystals that precipitated out were collected on a sintered-glass filter. Recrystallization from 0.1 M HCl solution gave pale yellow needles of Pt(daba)Cl_2.

Equimolar amounts of dasa-ester·2HCl and K_2PtCl_4 were dissolved in water and then the solution was adjusted to pH 5.0—6.0 with $NaHCO_3$. After standing for a day at room temperature, the pale yellow crystals that precipitated out were collected and recrystallized from water containing NaCl. Pale yellow needles of Pt(dasa-ester)Cl_2 were obtained. Pt(dapa-ester)Cl_2 and Pt(daba-ester)Cl_2 were prepared by similar procedures.

Table I shows analytical and infrared spectral data for these platinum complexes. The infrared spectra showed $\nu_{C=O}$ at 1700—1750 cm^{-1}. A protonated carboxyl group gives a $\nu_{C=O}$ frequency higher than 1700 cm^{-1}, while coordination of the carboxyl group to platinum moves the $\nu_{C=O}$ frequency below 1700 cm^{-1}.[6] Therefore, the carboxy groups of the platinum complexes obtained in this work are not involved in binding with platinum.

Table I. Analytical and Infrared Data for Platinum Complexes

	Analytical data	Analysis (%) Calcd C	H	N	Found C	H	N	Infrared data
Pt(dasa)Cl_2	$C_4H_8Cl_2N_2O_4Pt$	11.6	1.9	6.8	11.5	2.0	6.7	$\nu_{C=O}$ 1720, 1708; δ_{NH_2} 1575
Pt(dasa-ester)Cl_2	$C_8H_{16}Cl_2N_2O_4Pt$	20.4	3.4	6.0	20.4	3.3	6.0	$\nu_{C=O}$ 1750, 1735; δ_{NH_2} 1600
Pt(dapa)Cl_2	$C_3H_8Cl_2N_2O_2Pt$	9.7	2.2	7.6	10.1	2.2	7.2	$\nu_{C=O}$ 1757; δ_{NH_2} 1590
Pt(dapa-ester)Cl_2	$C_5H_{12}Cl_2N_2O_2Pt$	15.1	3.0	7.0	15.1	3.1	7.1	$\nu_{C=O}$ 1735; δ_{NH_2} 1565
Pt(daba)Cl_2	$C_4H_{10}Cl_2N_2O_2Pt$	12.5	2.6	7.3	12.4	2.5	7.0	$\nu_{C=O}$ 1715; δ_{NH_2} 1588
Pt(daba-ester)Cl_2	$C_6H_{14}Cl_2N_2O_2Pt$	17.5	3.4	6.8	17.4	3.3	6.7	$\nu_{C=O}$ 1732, 1713; δ_{NH_2} 1602

Interaction of the Platinum Complexes with DNA——Calf thymus DNA (type 1) was purchased from Sigma Chemical Co., U.S.A. DNA stock solutions were prepared by dissolving DNA in 0.01 M phosphate buffer, pH 6.8, containing 0.01 M NaCl by gentle stirring in a refrigerator. The concentration of the DNA solution was determined by spectrophotometry, taking $\varepsilon_{(p)}=6600$. The platinum complexes were dissolved before use in 0.01 M phosphate buffer, pH 6.8, containing 0.01 M NaCl.

An aliquot of platinum complex solution was added to the DNA solution and the mixture was incubated at 30° for 4 days. The amount of the platinum complexes added to the DNA solution is expressed as r, the molar ratio of platinum to phosphorus in DNA. In the measurement of UV difference spectra, the sample cell contained solutions of various r values ($r=0$—0.2), and the reference cell contained DNA solution. The UV difference spectra were recorded on a Shimadzu UV-200 spectrophotometer and were corrected in terms of the spectrum at $t=0$ (time of reaction) in order to avoid instrumental and absorption errors, which should be negligibly small under these conditions.

6) K. Nakamoto, "Infrared and Raman Spectra of Inorganic and Coordination Compounds," John Wiley and Sons, New York, 1978, p. 305.

Results and Discussion

Interaction of the Platinum Complexes with DNA

Figure 1a—1c show the UV difference spectra for DNA solution treated with the platinum complexes prepared in this work. All of the spectra exhibited an absorption maximum at 272 nm, a shoulder at 295 nm, and an absorption minimum at 248 nm. These UV difference spectral patterns are quite similar to each other. Figure 1d shows the UV difference spectra for DNA solution treated with $[Pt(NH_3)_3Cl]Cl$, *cis*-$Pt(NH_3)_2Cl_2$ or *trans*-$Pt(NH_3)_2Cl_2$. It is apparent that the UV difference spectral pattern for the *cis*-$Pt(NH_3)_2Cl_2$ is similar to those of Figs. 1a—1c. This UV difference spectral pattern is commonly observed for all antitumor active platinum complexes having *cis*-geometry, such as Pt(ethylenediamine)Cl_2, *cis*-$Pt(NH_3)_2Cl_2$, and Pt(1,2-cyclohexanediamine)Cl_2,[7] and is characteristic of the interaction of the platinum complexes having *cis*-geometry with DNA. Figure 2 shows the change of ΔA as a function of r. In every case, ΔA at 272 nm and 295 nm increased with increasing r, and there is a linear relationship between r ($r<0.1$) and ΔA_{272} or ΔA_{295}. The absorption ratio, $\Delta A_{272}/\Delta A_{295}$, was about 2, which is the same as that found in the *cis*-$Pt(NH_3)_2Cl_2$–DNA system.[7] In the case of the non-charged complexes (Figs. 2a, 2c, and 2e), ΔA_{248} decreased with increasing r until about $r=0.07$ and then it became constant. ΔA_{248} decreased with increasing r in the case of the charged complexes, and its behavior is significantly similar to that of the non-charged complexes until about $r<0.07$.

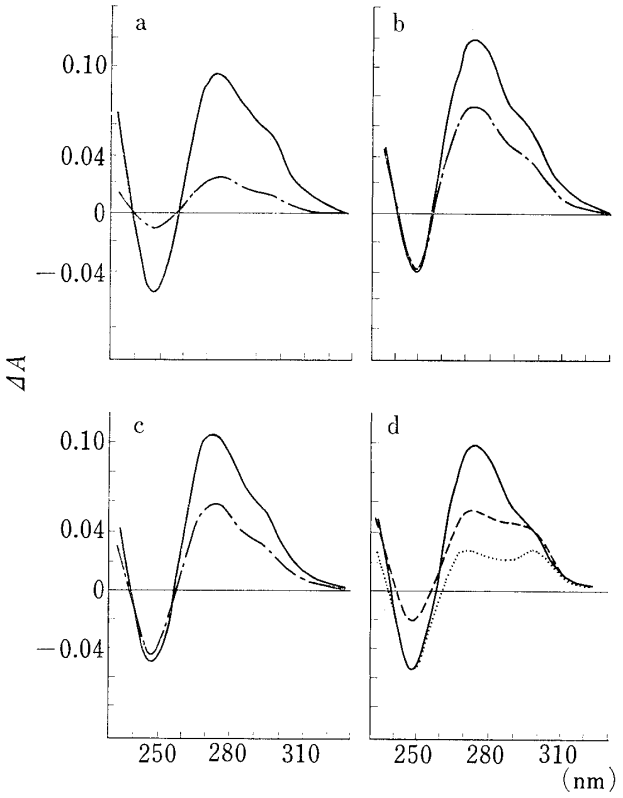

Fig. 1. Ultraviolet Difference Spectra for Various Platinum-DNA Systems ($r=0.1$) at pH 6.8

[DNA]$=2.36\times10^{-4}$ M, $t=4$ days, temp.$=30°$.
a) ——: Pt(dasa-ester)Cl_2, – – –: Pt(dasa)Cl_2
b) ——: Pt(dapa-ester)Cl_2, – – –: Pt(dapa)Cl_2
c) ——: Pt(daba-ester)Cl_2, – – –: Pt(daba)Cl_2
d) ——: *cis*-$Pt(NH_3)_2Cl_2$, – – –: *trans*-$Pt(NH_3)_2Cl_2$
····: $[Pt(NH_3)_3Cl]Cl$.

We will next consider the assignment of the absorption bands at 272, 295, and 248 nm. It is likely that the absorption at 295 nm is attributable to the change in electron distribution of the base moieties induced by the binding of platinum. The UV difference spectrum obtained by the interaction of Pt(dasa)Cl_2 with deoxyguanosine showed a maximum at 292 nm and a minimum at 248 nm, and this spectral pattern is quite similar to that the *cis*-$Pt(NH_3)_2Cl_2$-guanosine system reported by Scovell and O'Connor.[8] It seem, therefore, that the absorptions at 295 nm and 248 nm in Figs. 1 and 2 reflect the binding of the platinum complexes with guanine residues of DNA. The absorption at 295 nm tends toward saturation with increasing r ($r>0.2$), though it did not become completely constant. Tobias *et al.* reported that *cis*-$Pt(NH_3)_2Cl_2$ bound with high selectivity to the guanine residues in calf thymus DNA at $r<0.2$,

7) K. Inagaki and Y. Kidani, *Inorg. Chim. Acta*, **46**, 35 (1980).
8) W.M. Scovell and Y. O'Connor, *J. Am. Chem. Soc.*, **99**, 120 (1977).

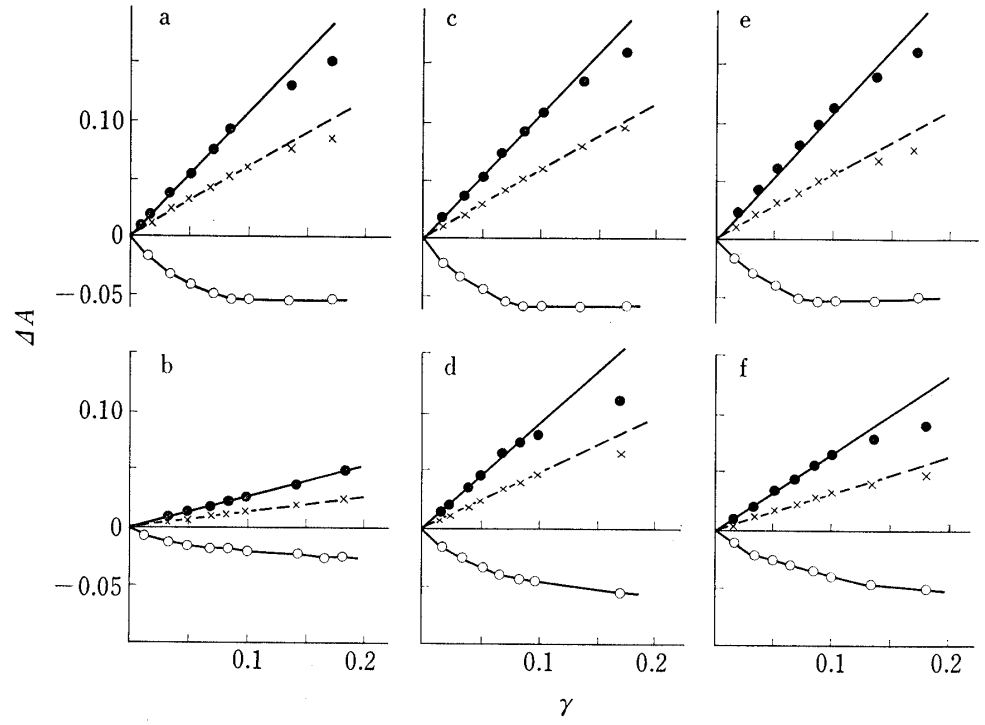

Fig. 2. Variation of A as a Function for Various Platinum–DNA Systems at pH 6.8

—×—: A at 295 nm, —●—: A at 272 nm, —○—: A at 248 nm.
a) Pt(dasa-ester)Cl_2, b) Pt(dasa)Cl_2, c) Pt(dapa-ester)Cl_2, d) Pt(dapa)Cl_2, e) Pt(daba-ester)Cl_2, f) Pt(daba)Cl_2.

using Raman difference spectroscopy.[9] Luck and Zimmer reported that the UV difference spectrum obtained by the interaction of Mn^{2+} with DNA showed a maximum at 294 nm; Mn^{2+} coordinated with the N(7) position of guanine residues, and this band was attributable to perturbation of the electron system of the DNA chromophore.[10]

It is considered that the absorption at 272 nm depends primarily on the change in the DNA secondary structure induced by binding with platinum. In the case of the UV difference spectra of *cis*-$Pt(NH_3)_2Cl_2$-nucleoside systems (guanosine, adenosine, and cytidine),[8] ΔA around 270 nm showed little change on the binding of platinum with nucleosides. It is well known that the absorbance around 260 nm of native DNA is affected by the interbase π-interaction, *e.g.*, heat denaturation of native DNA leads to the loss of base stacking and results in a hyperchromic effect. In the case of DNA-platinum, the higher the absorption at 270 nm, the lower the degree of hyperchromicity on melting.[7]

The absorption at 248 nm seems to be a result of changes in both the electron distribution of the guanine residues and the DNA secondary structure. If the platinum complexes binds only to the guanine residues of DNA and consequently only the electron distribution of the guanine base is changed, the absorption at 248 nm may correlate with that at 295 nm. However, this cannot be observed in the variation of ΔA as a function of r in Fig. 2, especially in the case of large r values. This may be because the absorption at 248 nm is influenced not only by the binding of platinum with guanine residues but also by the hyperchromicity induced by binding of platinum with DNA and by the binding of platinum with other bases (adenine and/or cytosine bases).

9) S. Mansy, G.Y.H. Chu, R.E. Duncan, and R.S. Tobias, *J. Am. Chem. Soc.*, **100**, 607 (1978).
10) G. Luck and C. Zimmer, *Eur. J. Biochem.*, **29**, 528 (1972).

From the similarity of the UV difference spectral patterns obtained for both the charged and non-charged complex-DNA systems, the binding mode of the charged complexes with DNA appears to be the same as that of the non-charged complexes, even though the value of ΔA is different. Assuming that the absorption at 295 nm depends on the extent of platinum binding to DNA bases, presumably guanine base, it is clear from Fig. 1 and 2 that the presence of negative charge lowers the reactivity of the platinum with DNA. The reactivities of the non-charged complexes (Pt(dasa-ester)Cl_2, Pt(dapa-ester)Cl_2 and Pt(daba-ester)Cl_2) are almost the same. Pt(dasa)Cl_2 has a charge of -2 under physiological conditions, while Pt(dapa)Cl_2 and Pt(daba)Cl_2 have a charge of -1. The reactivity of the former is lower than those of the latter compounds. Therefore, the lowering of the reactivity due to the negative charge may be attributed to repulsion between the negative charge and the phosphate groups of DNA. This is also supported by the finding that the interactions of deoxyguanosine with Pt(dasa)Cl_2 and Pt(dasa-ester)Cl_2 show almost the same reactivity.

Antitumor Activity of the Platinum Complexes

The antitumor activities of the platinum complexes were evaluated against leukemia L-1210 and P-388 by *i.p.* administration. Table II shows that the non-charged complexes exhibited antitumor activity, but their activities were not especially high when compared with those of other platinum complexes such as Pt(1,2-cyclohexanediamine)Cl_2,[11,12] Pt(1,2-cyclopentanediamine)Cl_2,[13] and Pt(1-amino-2-aminomethylcyclohexane)Cl_2.[14] On the other hand, the charged complexes were antitumor-inactive. Although the reactivity of the charged complexes with DNA is lower than that of the non-charged complexes, the binding modes of both the charged and the non-charged complexes seem to be the same, because the UV difference spectral patterns are identical. However, Pt(dasa)Cl_2 is antitumor-inactive even at a dose of 200 mg/kg. This suggests that the cell permeability plays an important role in the appearance of the antitumor activity of the platinum complexes. It is therefore considered that the antitumor inactivity of the charged platinum complexes is due to the reduced ability to pass into the cells and to the lower reactivity with DNA compared with the non-charged platinum complexes.

Table II. Results of Antitumor Screening of Platinum Complexes of Diaminocarboxylates and Their Ethyl-ester Derivatives

Compd.	Dose, (mg/kg)/day Test system	200	100	50	25	12.5	6.25
				T/C, %			
Pt(dasa)Cl_2	P-388	97	101	117	100	96	96
Pt(dasa-ester)Cl_2	P-388	—	—	—	164	163	131
Pt(dasa)Cl_2	L-1210	104	106	100	—	—	—
Pt(dasa-ester)Cl_2	L-1210	—	—	—	195	186	137
Pt(dapa)Cl_2	L-1210	—	—	108	117	115	—
Pt(dapa-ester)Cl_2	L-1210	—	—	112	175	153	—
Pt(daba)Cl_2	L-1210	—	—	115	121	107	—
Pt(daba-ester)Cl_2	L-1210	—	—	93	210	182	—

P-388: 10^6 cells/mouse, *i.p.-i.p.*, CDF_1 mice (6 mice/group), administered on days 1 and 5.
L-1210: 10^5 cells/mouse, *i.p.-i.p.*, CDF_1 mice (6 mice/group), administered on days 1, 5, and 9.

In the antitumor screening of the platinum complexes of 1,2-diaminobenzene derivatives,[15] the non-charged complexes, Pt(1,2-diaminobenzene)Cl_2 and Pt(4-methyl-1,2-diamino-

11) Y. Kidani, K. Inagaki, and S. Tsukagoshi, *Gann*, **67**, 923 (1976).
12) Y. Kidani, K. Inagaki, M. Iigo, A. Hoshi, and K. Kuretani, *J. Med. Chem.*, **21**, 1315 (1978).
13) Y. Kidani, K. Inagaki, T. Yashiro, T. Tashiro, and S. Tsukagoshi, *Chem. Pharm. Bull.*, **27**, 829 (1979).
14) Y. Kidani, K. Okamoto, M. Noji, and T. Tashiro, *Gann*, **69**, 869 (1978).
15) Y. Kidani, Y. Asano, and M. Noji, *Chem. Pharm. Bull.*, **27**, 2577 (1979).

benzene)Cl_2 showed T/C values of 193% and 171% at a dose of 12.5 mg/kg, respectively. Even though it is a charged complex, Pt(1,2-diaminobenzene-4-sulfonic acid)Cl_2 showed a T/C value of 143% at a dose of 100 mg/kg. In this case, a higher dosage was required for the appearance of antitumor activity of the charged complex compared with the non-charged complexes. The appearance of activity of the charged complexes may be due to a lower charge effect relative to the size of molecule. This is consistent with the finding that the binding mode of the charged complexes with DNA may be the same as that of the non-charged complexes.

Schwartz *et al.*[16] reported that 4-carboxyphthalato(1,2-cyclohexanediamine) platinum, which is readily soluble in 1% sodium bicarbonate, had a high antitumor activity against leukemia L-1210. This complex is a charged complex, having a negative charge on it leaving group. Cleare has proposed that an *in vivo* activation mechanism might be operating for platinum complexes containing bidentate carboxylate as a leaving ligand.[17] A charge on the non-leaving ligand clearly has a disadvantageous effect on the antitumor activity, while a charge on the leaving ligand, if the leaving ligand is replaced by a suitable species *in vivo*, may aid in the solubilization of antitumor active platinum complexes.

16) P. Schwartz, S.J. Meishen, G.R. Gale, L.M. Atkins, and A.B. Smith, *Cancer Treat. Rep.*, **61**, 1519 (1977).
17) M.J. Cleare, P.C. Hydes, B.W. Malerbi, and D.M. Watkins, *Biochimie*, **60**, 835 (1978).